Methicillin Resistance Reduces the Virulence of Healthcare-Associated Methicillin-Resistant *Staphylococcus aureus* by Interfering With the *agr* Quorum Sensing System

Justine K. Rudkin,1 Andrew M. Edwards,1 Maria G. Bowden,2 Eric L. Brown,3 Clarissa Pozzi,4 Elaine M. Waters,4 Weng C. Chan,5 Paul Williams,6 James P. O’Gara,4 and Ruth C. Massey1

1Department of Biology and Biochemistry, University of Bath, United Kingdom; 2Center for Infectious and Inflammatory Diseases, Texas A&M Health Science Center, 3The University of Texas School of Public Health, Houston; 4School of Biomolecular and Biomedical Science, University College Dublin, Ireland; 5School of Pharmacy, and 6School of Molecular Medical Sciences, Centre for Biomolecular Sciences, University of Nottingham, United Kingdom

The difficulty in successfully treating infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) has led to them being referred to as highly virulent or pathogenic. In our study of one of the major healthcare-associated MRSA (HA-MRSA) clones, we show that expression of the gene responsible for conferring methicillin resistance (*mecA*) is also directly responsible for reducing the ability of HA-MRSA to secrete cytolytic toxins. We show that resistance to methicillin induces changes in the cell wall, which affects the bacteria’s *agr* quorum sensing system. This leads to reduced toxin expression and, as a consequence, reduced virulence in a murine model of sepsis. This diminished capacity to cause infection may explain the inability of HA-MRSA to move into the community and help us understand the recent emergence of community-associated MRSA (CA-MRSA). CA-MRSA typically express less penicillin-binding protein 2a (encoded by *mecA*), allowing them to maintain full virulence and succeed in the community environment.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infections worldwide, with factors such as exposure to broad-spectrum antibiotics, presence of indwelling catheters, and recent surgery leading to increased susceptibility to infection [1–3]. Genetic analyses of healthcare-associated MRSA (HA-MRSA) have shown that certain clones have become endemic in these environments, but these MRSA are rarely found to cause infections in individuals with no healthcare contact (ie, in the community environment [2, 3]). The recent emergence of community-associated MRSA (CA-MRSA), which can cause infections outside healthcare settings among otherwise healthy individuals, is a growing concern [2, 3]. Comparisons of HA-MRSA with CA-MRSA have revealed genetic differences between these 2 groups [3–6] but none that explain why HA-MRSA is restricted to healthcare environments when CA-MRSA is not.

Resistance to methicillin and oxacillin (the clinically used derivative of methicillin) is conferred by the acquisition of a mobile genetic element: staphylococcal chromosomal cassette *mec* (SCC*mec*) [4]. The elements vary in size (20–70 kb) and genetic content, but they all contain the *mecA* gene, which encodes an alternative penicillin-binding protein (PBP), PBP2a [5, 6]. Genetic analyses of HA-MRSA and CA-MRSA have shown that HA-MRSA tends to carry the large types I, II, and III SCC*mec* elements, whereas CA-MRSA tends to carry the smaller type IV and V elements [5, 6]. Penicillin-binding proteins are membrane-bound enzymes that catalyze the
transpeptidation and transglycosylation of peptidoglycan, the major structural component of the bacterial cell wall [6]. β-Lactam antibiotics irreversibly acylate the catalytic serine within the transpeptidase active site of PBPs [7]. This impairs effective peptidoglycan cross-linking, destabilizing the cell wall and resulting in bacterial lysis. Methicillin-resistant S. aureus strains are resistant because PBP2a has a lower affinity for β-lactam antibiotics, enabling it to continue its catalytic activity even in the presence of the antibiotic [7]. However, the expression of PBP2a alone is often not sufficient to confer resistance; many other contributing cell wall and membrane proteins have been identified [8–10].

Studies comparing the relative virulence of MRSA types and methicillin-susceptible S. aureus (MSSA) suggest a contradictory virulence hierarchy: HA-MRSA > MSSA [11–13]; CA-MRSA > HA-MRSA [14, 15] and MSSA = CA-MRSA [16, 17]. This illustrates the need for further characterization of their relative virulence. The environments in which these infections occur will select for the most successful pathogen. An understanding of how (and if) the relative virulence of these strains vary depending on environment will inform us of strategies to employ to control and contain their success.

We previously reported that MRSA strains containing the hospital-associated type II SCCmec element were less toxic (as measured by their ability to lyse T cells) than their antibiotic-sensitive relatives, whereas the toxicity of strains carrying the smaller community-associated type IV elements were not affected [18, 19]. Here we demonstrate that the reduced toxicity of HA-MRSA is a direct result of mecA expression, mediated by interference with the S. aureus accessory gene regulator (agr) quorum sensing system. We found that this MRSA was impaired in its ability to respond to the agr auto-inducing peptide (AIP), but removal of the cell wall restored this ability. This suggests that the expression of mecA and either its potential to subtly affect peptidoglycan structure or its interaction with other cell wall–associated proteins prevent the AIP from being detected. This results in an unresponsive agr system and subsequent low-level toxicity. To compare the virulence of MRSA to its isogenic MSSA strain, we used a murine infection model and found that the expression of the antibiotic-resistant gene mecA reduced the virulence of MRSA. As a direct consequence of its high level of antibiotic resistance, HA-MRSA is impaired in its ability to cause infection, which can explain its inability to cause infection in community settings, where antibiotic usage and the prevalence of susceptible patients are low.

**MATERIALS AND METHODS**

**Strains and Plasmids Used in This Study**

**Construction of mecA Mutation in BH1CC**

The mecA gene was amplified by polymerase chain reaction (PCR) from BH1CC using Phusion high-fidelity DNA polymerase (NEB) and cloned into PCR-Blunt II-TOPO plasmid (Invitrogen) and inactivated by cloning the tetracycline resistance gene from plBlue::tet into its internal MfeI-NheI sites (Table 1). This was subcloned into a temperature-sensitive *Escherichia coli–Staphylococcus* shuttle vector pBT2-hga and transformed by electroporation into *S. aureus* RN4220 and finally into *S. aureus* MRSA BH1CC. Allelic replacement of the temperature-sensitive pSAmeC8 in BH1CC was achieved following repeated growth (3 subcultures) at 42°C for 24 hours without antibiotic selection, followed by selection of tetracycline-resistant (10 µg/mL) colonies on brain-heart infusion (BHI) agar plates. The colonies were then screened for chloramphenicol sensitivity to confirm plasmid loss, and PCR analysis was used to verify the presence of the mecA::Tcr allele on the chromosome. The mutation was confirmed by Western immunoblot analysis of PBP2a expression and reduced oxacillin minimum inhibitory concentration (MIC; data not shown).

**Cloning and Expression of mecA and Construction of the S403A Mutation**

Controlled expression of mecA was achieved using a tetracycline-inducible system previously described [27]. The mecA coding region was amplified by PCR using the primers TTTGTTGACGTTTATATATAAGGAGGATTTGATG and AAAGAGCTCCGTATCGCTGAATTAC and cloned into plasmid pRMc2 to make pmeC. This was electroporated into *S. aureus* RN4220 and subsequently into *S. aureus* BH1CC Δscmc to complement the mecA mutation. A serine-to-alanine substitution at position 403 (critical catalytic codon [7]) was made in pmeC using the primers GTGAAGTGTGATTCTGGAATTCTTTTGAGC and CAGGTGCAACTCAAAAATATTTACGCAATG in an inverse PCR approach, followed by ligation and transformation into DH5α to make pS403A. Mutation was verified by sequencing and reduced MIC to oxacillin, and the plasmid was transformed into BH1CCΔscmc, as described above.

**Construction of Oxacillin Resistant 8325-4**

Although tetracycline-induced expression of mecA from the plasmid pmeC was sufficient to restore oxacillin resistance to BH1CCΔscmc, it was not sufficient to confer oxacillin resistance to the MSSA strain 8325-4 (determined using Etest strips [Biomerieux]). Instead the mecA gene was recloned under the control of its own promoter into the shuttle vector pRB474 [22]. The mecA gene was amplified using primers SAmecA3 (TGAGCATCCAATGAGCAAC) and SAmecA4 (GCATCTGGAATTACGCAATG) and cloned initially into pCR-Blunt II-TOPO (pSAmecA5) and subsequently into an EcoRI fragment into pRB474 to create pmeC. The MSSA strain 8325-4 was transformed with pmeC and homogeneously resistant 8325-4 colonies were selected on BHI agar containing 100 µg/mL of oxacillin.

**T-Cell Toxicity Assays**

T-cell toxicity assays were performed as described previously [18, 30]. Where relevant, 200 ng/mL anhydrous tetracycline was...
added to induce expression from the tetracycline-inducible promoter p\text{mecA}
and pS403A.

**Growth Curves and RNAIII::GFP Readings**

BHI broth (100 mL in 250-mL conical flasks) was inoculated with 100 μL of overnight cultures (grown in BHI at 37°C, shaking at 180 rpm). Cultures were grown at 37°C, 180 rpm; samples were taken every hour; and optical density of 600 nm (OD\text{600}) was measured. Fluorescence intensity of samples was measured using a FARcyte XFLURO4 V 4.50 plate reader between 485 nm and 535 nm.

**RNA Isolation**

BHI broth (100 mL in 250-mL conical flask) was inoculated with 100 μL of overnight cultures and grown at 37°C, 180 rpm. RNA was isolated after 20 hours of growth using the Qiagen RNeasy Midi Kit according to the manufacturer’s instructions with an addition of 0.2 μg/μL of lysostaphin to the lysis step. RNA quality and concentration were determined using the Bio-Rad Experion RNA Analysis System.

**Reverse Transcription and qRT-PCR**

Complementary DNA (cDNA) was generated from messenger RNA (mRNA) using the NEB First Strand Synthesis kit in accordance with the manufacturer’s instructions using random hexamers. The following gyrB and RNAIII primers were used—RNA III forward: GAAGGAGTATTTCAATG GCACAAG; and reverse: GAAAGTATTAATTATCATCT TATTTTTAGTGAATTG; gyrB forward: CCAGGTAAAT

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**Table 1. Strains and Plasmids Used in the Study**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant Characteristics</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BH1CC</td>
<td>Clinical isolate, type II MRSA CC8</td>
<td>[19]</td>
</tr>
<tr>
<td>BH1CC\DeltaSCC\text{mec}</td>
<td>SCC\text{mec} excised from the chromosome via the recombinase activity of the \text{ccr}A and \text{ccr}B genes encoded on the plasmid pSR\text{2}</td>
<td>[18]</td>
</tr>
<tr>
<td>BH1CC\Delta\text{mecA}</td>
<td>Constructed via allele replacement to produce a Δ\text{mecA}::\text{Tcr} mutant. Tetracycline resistant</td>
<td>This study</td>
</tr>
<tr>
<td>BH1CC\DeltaSCC\text{mec} + p\text{mecA}</td>
<td>SCC\text{mec} mutation complemented with the \text{mecA} gene under tetracycline-inducible control</td>
<td>This study</td>
</tr>
<tr>
<td>BH1CC \DeltaSCC\text{mec} + pS403A</td>
<td>As above with a serine to alanine substitution at position 403 in the \text{mecA} gene. Serine residue responsible for cross-linkage of \text{PBP2a}</td>
<td>This study</td>
</tr>
<tr>
<td>RN4220</td>
<td>Restriction negative derivative of 8325-4</td>
<td>[20]</td>
</tr>
<tr>
<td>RN6390B</td>
<td>\text{agr} positive control strain</td>
<td>[21]</td>
</tr>
<tr>
<td>RN6911</td>
<td>\text{agr} negative derivative of RN6390B</td>
<td>[22]</td>
</tr>
<tr>
<td>8325-4</td>
<td>Laboratory oxacillin/methicillin-sensitive \text{Staphylococcus aureus} strains</td>
<td>[23]</td>
</tr>
<tr>
<td>8325-4 (p\text{mecA2})</td>
<td>Oxacillin resistant</td>
<td>This study</td>
</tr>
<tr>
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<td>MRSA (type II SCC\text{mec})</td>
<td>[24]</td>
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<td>[26]</td>
</tr>
<tr>
<td>SwedenAO</td>
<td>MRSA (type IV SCC\text{mec})</td>
<td>[25]</td>
</tr>
</tbody>
</table>

**Plasmids**

- p\text{RMC2} Plasmid containing a tetracycline-inducible promoter system | [27] |
- p\text{mecA} p\text{RMC2} plasmid containing the \text{mecA} gene under the control of a tetracycline-inducible promoter | This study |
- pS403A p\text{RMC2} plasmid containing the \text{mecA} gene under the control of a tetracycline-inducible promoter with a serine-to-alanine substitution at position 403 | This study |
- pRB474 \text{Escherichia coli}—\text{Staphylococcus} shuttle vector | [28] |
- p\text{mecA2} pRB474 containing the \text{mecA} under the control of its own promoter | This study |
- p\text{RNAIII::GFP} Plasmid containing a fusion of RNA III to GFP | [29] |

Abbreviations: GFP, green fluorescent protein; MRSA, methicillin-resistant \text{Staphylococcus aureus}. 

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The text above is formatted according to the guidelines provided. The tables and figures are represented as text, and the equations are not included in the natural text representation.
TAGCCGATTGC; and reverse: AAATCGCCTGCGTTCTAG AG. Standard curves were performed for each primer set on serial dilutions of cDNA to determine primer efficiency. The quantitative reverse-transcriptase PCR (qRT-PCR) reactions were set up as follows: 5 µL cDNA, 7.5 µL of SYBR reagent, 0.5 µL forward primer (10 µM), 0.5 µL reverse primer (10 µM), and RNase-free water to a total volume of 15 µL. Cycle threshold values were subsequently determined for 3 biological repeats in triplicate. For each reaction, the ratio of RNAIII and gyrB transcript number was calculated as follows:

$$2^{\Delta \Delta C_t}$$

**AIP Induction Assay**

Synthetic AIP-1 [31] was reconstituted in dimethyl sulfoxide (DMSO) to a concentration of 10 mM. Bacterial strains were grown overnight in BHI and used to inoculate 5 mL BHI (10 µg/mL of chloramphenicol) in 30-mL tubes. The bacteria were grown for 2–3 hours to an OD₆₀₀ of 0.2. The bacteria were washed and resuspended in 30% raffinose and 10% BHI. This medium was sufficient to maintain spheroplast integrity while providing sufficient nutrients for RNAIII transcription and GFP translation to take place. To each strain, combination of AIP-1, DMSO and lysostaphin (0.1 µg/mL) were added and incubated at 37°C for 3 hours. RNAIII::GFP expression was measured as described above every 30 minutes.

**Animal Experiment**

Casein casein yeast broth (10 mL in 50-mL flask) was inoculated with 200 µL of overnight cultures and grown at 37°C, 225 rpm to exponential phase (OD₆₀₀ = 0.8), harvested by centrifugation, washed, and resuspended in sterile phosphate-buffered saline to the desired infection dose. Bacterial density in the inoculum was verified by culturing serial dilutions on tryptic soy agar plates.

For the low inoculum infections, the mice were injected intravenously with 3–5 × 10⁶ S. aureus in a volume of 500 µL. Mice were weighed daily for 2 weeks, and weight change differences were analyzed for statistical differences using the Student t test. For the high inoculum infections, the mice were injected intravenously with 1–2 × 10⁷ S. aureus in a volume of 500 µL. Mortality was assessed up to 7 days after infection; statistical differences between groups were analyzed using the Student t test. All surviving mice were euthanized at day 7 after inoculation.

Criteria for determining morbidity/sickness in mice included hunched posture, decreased activity, ruffled fur, and labored breathing. All animal experiments conformed to the National Institutes of Health guidelines and were approved by the Institutional Animal Care Use Committee at the Texas A&M Health Science Center Institute of Bioscience and Technology.

**Measurement of MIC to Oxacillin**

Measurement of MIC to oxacillin was performed in accordance with the Clinical and Laboratory Standards Institute guidelines using Etest strips from Biomérieux on Mueller Hinton agar containing 2% sodium chloride.

**Analysis of PBP2a Expression**

Total cell protein preparations were made from overnight cultures of bacteria grown in BHI. The cells were harvested and lysed in water containing 10 µg/mL lysostaphin, 4 units of DNAase, 4 units of RNAase, and 0.05 mg/mL sodium dodecyl sulphate by incubating at 37°C for 1 hour. Insoluble material was removed by centrifugation, and protein concentrations were determined by Bradford assay. Western blots were performed using anti-PBP2a antibodies (Abnova) and ProteinG-HRP (Sigma) and visualized using a colorimetric detection system (4CN, Bio-Rad). Band intensity was quantified using the ImageJ software (http://rsbweb.nih.gov/ij/) and is expressed relative to the positive control run on each gel.

**RESULTS**

**Expression of mecA Reduces Toxicity**

In previous work, we reported that clinical MRSA isolates (n = 24) carrying the type II SCCmec element were less toxic than clonally matched MSSA strains [30]. We subsequently demonstrated that the carriage of the type II element was directly responsible for this by deleting it from the hospital-acquired MRSA strain BH1CC and showing that it restored cytolysis activity to MSSA levels [18]. While attempting to identify the gene(s) on this SCCmec element responsible for this phenotype, we found that deletion of the mecA gene alone was sufficient to restore toxicity (Figure 1A). Expression of the mecA gene from a tetracycline-inducible promoter (pntecA) in the BH1CCASCmec strain reduced toxicity to wild-type MRSA levels in a dose-dependent manner (Figure 1A). (The abundance of antibiotic resistance markers in the BH1CCASCmecA mutant prevented us from complementing mecA in this strain.) By contrast, expression of a mutant of mecA encoding a catalytically inactive PBP2a (expressed on p5405A [7]) failed to reduce toxicity. This effect was additionally verified by expressing oxacillin resistance in an MSSA strain (8325-4), in which the ability of the resistant strain to lyse T cells was reduced (Figure 1B).

**Expression of the agr-Regulated β-Toxin Is Affected by the Presence of mecA**

The Agr quorum sensing system is a major regulator of toxin expression in S. aureus [20], and several studies have reported a high incidence of agr dysfunction among clinical HA-MRSA strains based on their hemolytic profiles [30, 32]. The immortalized T cells used in this assay are sensitive to β-toxin [30], which is regulated by agr [33]. The functional expression of β-toxin by BH1CC, BH1CCASCmec, and BH1CCASCmecA was determined by hot-cold lysis of sheep blood [34], in which we found the expression of β-toxin to be lower in the MRSA strain BH1CC compared with its MSSA mutants (data not shown).
Deletion of the entire SCCmec element had a more significant effect than deletion of mecA alone, perhaps as a consequence of the additional regulatory elements found on these SCCmec elements [35, 36]. To verify that the observed effect on toxicity was not a result of adventitious mutations within the agr locus [37, 38], we sequenced it in BH1CC, BH1CC∆SCCmec, and BH1CC∆mecA. We found them to be identical to each other and to that of strain Newman, which has a functional agr system.

**Induction of the agr Quorum Sensing System Is Affected by the Presence of mecA**

We have previously shown that T cells are less susceptible to lysis when incubated with the supernatant of an agr mutant [30]. To examine whether altered agr activity contributed to the reduced MRSA toxicity, we introduced a plasmid containing the RNAIII promoter fused to the green fluorescent protein (RNAIII::GFP [29]) into BH1CC, BH1CC∆SCCmec, BH1CC∆mecA, and isogenic control strains RN6390B (agr1 [21]) and RN6119 (agr2 [22]). No differences in the ability of these strains to grow was detected by measuring the OD600 over a growth curve (data not shown); however, their ability to induce expression of RNAIII was affected by the presence of the mecA gene (Figure 2). The MRSA strain BH1CC and the agr2 strain RN6119 did not display the typical stationary phase RNAIII induction, whereas deletion of the SCCmec element or mecA alone restored this induction to levels similar to the control agr+ strain RN6390B. The data is presented as the mean of five independent replicates, and the error bars are the 95% confidence intervals.
MRSA Cell Wall Prevents RNAIII Induction by Extracellular agr AIP

To switch on toxin expression, a threshold density of the secreted agr AIP is sensed, and this triggers expression of the agr regulatory effector-molecule RNAIII [21, 22]. That the MRSA strain BH1CC is unable to switch on the agr quorum sensing system, despite reaching high cell densities, suggested that it may be unable to respond to extracellular AIP. To test this, we developed an AIP induction assay by growing cells to early exponential phase in which RNAIII is not expressed. Synthetic AIP was added to these cells, and the induction of early exponential phase in which RNAIII is not expressed. The ability of strains to respond to AIP is represented as the percentage RNAIII expression relative to the agr control strain RN6390B 180 minutes after the addition of AIP. Whole cells of the MRSA strain BH1CC displayed significantly lower levels of RNAIII expression compared with the agr strain in response to the AIP, whereas the deletion of the entire SCCmec element or just mecA alone restored the ability of the strain to respond. The removal of the cell wall by digestion with lysostaphin (spheroplasts) enabled the MRSA strain BH1CC to respond to the AIP ($P = .0002$) but had no effect on the other strains. The asterisks indicate statistically significant differences ($<.05$).

Figure 3. The methicillin-resistant *Staphylococcus aureus* (MRSA) cell wall prevents the detection of the agr quorum sensing system auto-inducing peptide (AIP). A. Induction of RNAIII expression by the agr$^+$ strain RN6390B as measured by green fluorescent protein fluorescence over time in response to either synthetic AIP (squares) or dimethyl sulfoxide (solvent control, circles). B. The ability of strains to respond to AIP is represented as the percentage RNAIII expression relative to the agr$^+$ control strain RN6390B 180 minutes after the addition of AIP. Whole cells of the MRSA strain BH1CC displayed significantly lower levels of RNAIII expression compared with the agr$^+$ strain in response to the AIP; whereas the deletion of the entire SCCmec element or just mecA alone restored the ability of the strain to respond. The removal of the cell wall by digestion with lysostaphin (spheroplasts) enabled the MRSA strain BH1CC to respond to the AIP ($P = .0002$) but had no effect on the other strains. The asterisks indicate statistically significant differences ($<.05$).

**The mecA Gene on the Type II SCCmec Element Reduces Virulence**

To directly compare the virulence of an isogenic set of MRSA and MSSA strains, and to verify that the effect of the mecA gene on the toxicity of a strain is not an in vitro phenomenon, we compared BH1CC, BH1CCΔSCCmec, and BH1CCΔmecA in a murine sepsis model. The relative weight loss of the mice infected with these strains can be seen in Figure 4A, and the percentage survival of the mice can be seen in Figure 4B. These data show a significant difference in morbidity and mortality between these isogenic MRSA and MSSA strains, demonstrating that the presence of the mecA gene significantly reduces the virulence of this MRSA strain.

**MRSA Strains Carrying the Type IV SCCmec Element Express Less PBP2a and Are More Toxic Than Those Carrying the Type II Element**

We previously reported that MRSA strains carrying the community-associated type IV SCCmec element were as toxic as MSSA strains [18, 30]. We hypothesized that because...
these strains tend to be less resistant to oxacillin than HA-MRSA [41], they may express lower levels of PBP2a and, as a consequence, may be able to fully express toxins. To test this, we compared the toxicity, the MIC of oxacillin, and the expression of PBP2a of a set of clinical MRSA strains carrying either the hospital-associated type II or the community-associated type IV SCCmec element. As presented in Table 2 and Figure 5, those carrying the type II SCCmec element all had an MIC of greater than 256 μg/mL to oxacillin, a T-cell survival rate between 28.6% and 88%, and a relatively high level of expression of PBP2a, whereas those carrying the type IV SCCmec element had lower MICs (12–32 μg/mL) and higher toxicity (T-cell survival, 0%–13%) and expressed less PBP2a (on average 13-fold less). This lower level of PBP2a expression may explain why MRSA strains carrying the type IV SCCmec elements, although oxacillin resistant, are more toxic than those carrying type II elements.

**DISCUSSION**

For HA-MRSA, the evolution of resistance to oxacillin has a clear selective advantage in environments where antibiotic usage is high. The secondary effect of a decrease in virulence associated with resistance has not undermined its ability to become a successful nosocomial pathogen. It is likely that the high prevalence of susceptible patients in these environments and an additional transmission route via healthcare workers, compensate for reduced virulence. The reduced toxicity is, however, a likely explanation for why these HA-MRSA

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**Table 2. Phenotypic Characteristics of Methicillin-Resistant Staphylococcus aureus Strains Carrying Type II and Type IV SCCmec Elements**

<table>
<thead>
<tr>
<th>Strain</th>
<th>SCCmec Type</th>
<th>T-Cell Survival, % (±95% CI)</th>
<th>Oxacillin MIC (μg/mL)</th>
<th>PBP2a Expression ( Arbitrary Relative Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA252</td>
<td>II</td>
<td>88 (1.4)</td>
<td>&gt;256</td>
<td>Not done</td>
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<tr>
<td>MRSA45</td>
<td>II</td>
<td>69.6 (15.4)</td>
<td>&gt;256</td>
<td>865</td>
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<td>MRSA137</td>
<td>II</td>
<td>45 (29)</td>
<td>&gt;256</td>
<td>3930</td>
</tr>
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<td>MRSA281</td>
<td>II</td>
<td>28.6 (8.2)</td>
<td>&gt;256</td>
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<tr>
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<td>66.6 (12.8)</td>
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<td>6588</td>
</tr>
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<td>MRSA504</td>
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<td>69.7 (8.4)</td>
<td>&gt;256</td>
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<td>71.5 (7.3)</td>
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<td>IV</td>
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</table>

Abbreviations: CI, confidence interval; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; PBP, penicillin-binding protein.
strains have not been successful outside hospital settings among healthy communities. The emergence of successful MRSA strains infecting healthy populations with no contact with healthcare settings (CA-MRSA) is a growing concern [2, 4, 14], and here we show that despite the presence of mecA on the type IV SCCmec elements, toxicity is not affected due to their lower level of PBP2a expression. This has allowed these strains to maintain both virulence and antibiotic resistance and succeed where HA-MRSA could not.

In addition to mecA, another putative virulence regulatory locus has been identified on the hospital-associated type II SCCmec element [36], as well as a cytolytic, phenol-soluble modulin, psm-mec [35]. Alongside our findings of the dual activity of mecA (antibiotic resistance and virulence regulation), this suggests that linkage between antibiotic resistance and virulence regulation has played an important role in shaping the success of this pathogen. Future in-depth studies on the molecular interactions of these regulatory systems with those already characterized (agr, sae, rot, etc) may fundamentally change our understanding of virulence regulation for this important human pathogen.

That an antibiotic resistance mechanism decreases the virulence of a pathogen complicates our understanding of pathogenicity and virulence. If we consider pathogenicity to be environment dependent, then the HA-MRSA strains are more pathogenic in hospitals because they can persist despite intense antibiotic pressure. In contrast, HA-MRSA strains are less pathogenic in the community because their impaired ability to express toxins is not compensated for by the benefits of being antibiotic resistant. This is reflected in their relative success and prevalence in these environments. Virulence, however, is independent of its environment, and in this study we show that HA-MRSA strains are less virulent as a direct consequence of expressing the mecA gene at high levels. This may seem an argument in semantics, but it is important to clearly understand these differences if we are to continue to study the means by which this pathogen causes disease in the hopes of identifying targets for the development of novel control and treatment strategies [42].

Notes

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References
